INFERENCES ABOUT DIETS BASED ON DELTA ^{13}C ANALYSIS OF COLLAGEN AND OTHER TISSUES FROM MODERN AND EARLY HISTORIC ANIMALS

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ABSTRACT

Stable isotopic analysis of bone collagen and other tissues provides a direct means for analyzing both modern and prehistoric animal diets. This procedure can supplement other more standard techniques, e. g., fecal and rumen analyses and behavioral studies, already in use for dietary analysis. Animals in the Great Plains have been greatly affected by agriculture. This becomes apparent when comparing prehistoric to modern animal diets. A prehistoric raccoon's $\delta^{13}C$ value is very negative -20.3°/$_{oo}$ when compared to a modern raccoon's value of -11.9°/$_{oo}$. This difference documents a shift from a C_3 diet to an agriculturally influenced C_4 diet. This shift is also seen in deer, where it is somewhat confounded by different isotopic signals for modern males and females. The more negative values for males suggests greater reliance on vegetation associated with forests and less on corn, relative to female deer. Other modern species showed variation in isotopic values among individuals, suggesting opportunistic and habitat specific feeding patterns.

INTRODUCTION

Wildlife management is often dependent on an understanding of each species' dietary requirements. In the past, diets have been determined largely by direct observation of behavior or analyzing rumen contents and fecal material (Tieszen et al., 1983a). Recently, a novel technique using stable isotopes for dietary analysis has been developed. This technique is based on using the two naturally occurring stable isotopes of carbon. Plants use two photosynthetic pathways (C_3 or C_4) that discriminate unequally between ^{12}C and ^{13}C, resulting in different isotopic ratios. The C_3 system uses the enzyme Ribulose bisphosphate carboxylase (Rubisco) to fix carbon dioxide into a three-carbon molecule. The C_4 system utilizes the enzyme Phosphoenolpyruvate carboxylase (PEPcase) to fix carbon dioxide

into a four-carbon molecule. Each system discriminates against ^{13}C differently, producing two distinct isotopic ratios. This ratio is expressed by the equation:

$$\delta^{13}C = \left[\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1 \right] X\ 1000\,^{o}/_{oo}$$

where the standard is carbonate in the fossilized shell of a belemnite from the Pee Dee formation in South Carolina (Boutton, 1984). C_4 plants, which discriminate less against the heavier ^{13}C show an average $\delta^{13}C$ value of $-12.5\,^{o}/_{oo}$ with an average range from $-9.0\,^{o}/_{oo}$ to $-16.0\,^{o}/_{oo}$. C_3 plants, which discriminate more against ^{13}C, show an average $\delta^{13}C$ value of $-27.0\,^{o}/_{oo}$ with a range from $-22.0\,^{o}/_{oo}$ to $-33.0\,^{o}/_{oo}$ (Lynott et al., 1986).

The isotopic ratio present in the food resource is transmitted along the food chain resulting in quantitatively detectable isotopic values in consumers (Klepinger, 1984). Each tissue and biochemical fraction may have its own distinct isotopic "memory" which is a function of the $\delta^{13}C$ ratio of carbon in the food at the time of its synthesis (Tieszen et al., 1983a). Carbon in these tissues, however, also turns over. This turnover rate reflects the metabolic activity of the tissue. Bone and connective tissue have slow turnover rates and therefore provide a good isotopic record of lifelong diet. Fatty tissue and muscle turn over faster and record recent diet (Tieszen et al., 1983a). Because animals do not substantially alter the carbon isotope composition of their food (DeNiro and Epstein, 1978), an herbivore's $\delta^{13}C$ value will reflect the relative amounts of C_3 and C_4 plants in the diet. Although some fractionation may occur as carbon is passed to a higher trophic level (Lee-Thorp et al., 1989), a carnivore's $\delta^{13}C$ value will reflect the signal of the tissue consumed and, ultimately, the proportions of C_3 and C_4 plants consumed by herbivores. Thus, aspects of an animal's diet can be reconstructed based on the $\delta^{13}C$ tissue value, some knowledge of feeding patterns, and an understanding of the C_3 and C_4 plants of its habitat. The degree of carnivory and herbivory can also be estimated using nitrogen isotopes which become enriched $3\,^{o}/_{oo}$ per trophic level (Schoeninger and DeNiro, 1983).

In this study we collected modern and early historic animal resources from the central Great Plains. Several tissues from both groups were analyzed for $\delta^{13}C$ and $\delta^{15}N$ values. These data provide important baseline information for the analysis of human and animal diets and, even with a small sample size, allow us to compare changes in dietary patterns from early historic to modern times.

MATERIALS AND METHODS

Modern animal resources were collected near Thorpe Park, five miles west of Forest City, in Winnebago County in north central Iowa. This area is characterized by an abundance of wetlands, forests and water bodies. Corn and soybeans are the predominant agricultural vegetation. Much of this area has also been set aside in the Conservation Reserve Program. All animals were either trapped or shot for other reasons in December of 1990.

Prehistoric or early historic animal resources were provided by the Anthropology Department of the University of Nebraska at Lincoln. Samples were recovered from a single village in the upper Dakota site along the Missouri River valley in the northeastern corner of Nebraska. The Dakota site has approximately 32 feet of loess and is predominantly tallgrass prairie with both C_3 and C_4 species. Samples are approximately 500-700 years BP based on associated artifacts from this site.

Samples of modern animal tissues were dried and loaded in tin cups for analysis. Modern and prehistoric collagen was isolated by treating cleaned bone with 0.5 M EDTA and/or 0.3 N HCl, depending on bone stability, until decalcified. Humic acids and other base soluble contaminates were removed by treating with 0.125 N NaOH for 12 hours. Pseudomorphs were placed in a methanol/chloroform/water solution (2:1:0.8) for 15-20 min. to remove lipids. Bone gelatin was purified by solubilizing in 90°C, acidic water (pH 3) and freeze-drying. Prehistoric apatite was prepared by treating cleaned ground bone on a shaker with 1.5% $NaHClO_4$ for 12 hours to remove organic materials. Treatment with 1N acetic acid for 48 hours removed adsorbed carbonate (Lee-Thorp and van der Merwe, 1987). The sample was then freeze-dried. Apatite CO_2 was released by reacting 150 mg apatite with 3 ml 85% phosphoric acid under vacuum. The sample was then cryogenically purified and collected with 1-propanol-dry ice and liquid nitrogen cold fingers. $\delta^{13}C$ and $\delta^{15}N$ were analyzed on a VG SIRA 10 Isotope Ratio Mass Spectrometer with a precision better than $0.2\,^{o}/_{oo}$ for carbon and better than $0.5\,^{o}/_{oo}$ for nitrogen.

RESULTS AND DISCUSSION

An array of tissues was analyzed for all modern animals; however, only collagen, muscle, and fat will be examined in detail. The two modern deer possess different collagen and different soft tissue $\delta^{13}C$ values (Table 1). Collagen ($-21.3\,^{o}/_{oo}$) values from the male deer show a definite C_3 signal approaching almost 100% C_3 dietary intake. This is supported by the negative value for fat ($-24.7\,^{o}/_{oo}$), although since fat is generally depleted in plants, this value could have been even

Table 1. Isotopic values (‰) for all modern wildlife samples taken from the North Central Iowa area, December 1990.

Scientific Name	Sex	C:N	$\delta^{13}C$ of Tissues							$\delta^{15}N$
			Collagen	Muscle	Fat	Hair	Skin	Tendon	Claw	
Odocoileus virginianus[1]	M	NA	-21.2	-19.2	-24.7	-19.9	-20.3	-20.9	-16.4	7.0
	F	NA	-15.5	-18.1	-19.6	-15.2	-15.3	-14.3	NA	7.2
Procyon lotor[2]	M	NA	-11.4	-12.8	-14.1	-12.1	-11.6	-12.2	-12.4	10.2
	F	NA	-12.4	-14.4	-15.4	-13.9	-14.1	-11.5	-12.8	11.3
Mustela vison[3]	M	NA	NA	-16.9	NA	-17.5	-16.8	NA	-15.0	12.3
	M	3.2	-16.3	-21.1	-23.7	-20.2	-21.4	-17.6	-18.5	10.3
Ondatra zibethica[4]	NA	3.3	-20.1	-22.8	-25.6	-20.1	-21.2	-18.6	-20.2	11.0
	NA	3.2	-16.6	-20.3	-21.8	-18.9	-20.5	-14.9	-18.8	10.1
Vulpes vulpes[5]	M	3.2	-17.8	-16.7	-19.5	-16.6	-16.4	-17.2	-15.5	9.8
	M	3.1	-13.9	-16.2	-22.2	-17.0	-16.6	-16.8	-17.6	9.9
Urocyon cinereoargenteus[6]	M	3.2	-16.0	-16.9	-23.0	-17.3	-17.7	-16.7	-17.4	11.1
Branta canadensis[7]	M	3.2	-19.4	NA	-21.3	NA	-20.2	-19.2	-16.3	10.1
Phasianus colchicus[8]	M	3.2	-11.1	NA	NA	-14.2	-13.0	-12.7	-12.8	8.2

Collagen was used for all nitrogen values except for the deer and the female raccoon where muscle tissue was used.

1 = Whitetail Deer, 2 = Raccoon, 3 = Mink, 4 = Muskrat, 5 = Red Fox, 6 = Grey Fox, 7 = Canadian Goose, 8 = Ringnecked Pheasant

more depleted (Tieszen et al., 1983b). Normally the pattern from a fully equilibrated animal would show muscle intermediate between collagen and fat (Tieszen et al., 1983b). The only way to explain muscle and hair values that are more positive than collagen is to conclude that the diet of this animal was more dependent on C_3 plants during the early years when collagen was formed, and that more recently the diet consisted of more C_4 input. Doe deer fat (-18‰), muscle (-19‰), and collagen (-15.5‰) have values indicating a recent mixed C_3 and C_4 diet and an early diet much richer in C_4 plants than the buck.

The large differences between the doe and buck likely reflect different habitat rather than food preferences. A buck is more secretive than a doe and, therefore, tends to spend more time in a wooded environment (Rue, 1979). Since most forest vegetation is C_3 (Ambrose, 1987), this may be the reason for its more negative signal. Both deer havefairly low $\delta^{15}N$ values, typical of herbivores (Ambrose and DeNiro, 1986). The raccoons give values that would be expected in an area dominated by modern corn-based agriculture. The buck raccoon collagen has a very positive signal of -11.4‰ and the sow raccoon shows a signal of -12.4‰. All other tissues are also very positive. These strong positive C_4 signals suggest that corn was a major component of their diets. The fairly high $\delta^{15}N$ value for both raccoons, however, is an indication of their partially carnivorous diets. Since both raccoons were trapped along a creek, these animal resources were likely frogs, mussels, or crayfish (Rue, 1964).

The high $\delta^{15}N$ values of the mink confirm carnivory. The $\delta^{13}C$ of the tissues of mink 1 indicate that this mink has been feeding on prey that ate C_4 vegetation. Mink 2 is more negative indicating that its prey utilized more C_3 plants. Mink will eat a diversity of prey including muskrats and waterfowl (Cahalane, 1947). Therefore, depending on the prey available in its habitat, the mink's diet and isotopic signal may vary considerably.

The muskrats also have a varied diet. The $\delta^{15}N$ for both muskrats is higher than would be expected but might be a result of their aquatic diet. With respect to carbon, tissues from muskrat 1 are more negative than the tissues of muskrat 2. These differences may be attributed to different habitats. The first was trapped in a dredge ditch and the second was trapped in a marsh with different vegetation.

The $\delta^{15}N$ values for the red fox suggest that these foxes were omnivorous. Red fox 1 showed a stronger signal for C_3 vegetation and animal matter that did red fox 2. The fat in red fox 2 (-22.2‰) is 8.3‰ more negative than collagen. This individual can be explained only by a large input of C_3 signal, which could account for the newly synthesized storage fat (Tieszen et al., 1983a). The tissues of the gray

fox also show that it had a recent change in diet. The more positive $\delta^{15}N$ for the gray fox shows that it was more carnivorous than the red fox.

The Canadian goose's tissues show a mixture of C_3 and C_4 plant material. This mixture is probably a result of migratory patterns and latitudinal vegetation changes (Tieszen, 1990). Collagen (-19.4°/$_{oo}$) documents the major C_3 input during growth and the fat (-21.3°/$_{oo}$), only 0.99°/$_{oo}$ more negative, shows a recent increase in the amount of C_4 material, e.g. corn, in the diet. In a fully equilibrated and constant diet, lipid values would be 4°/$_{oo}$ more positive than collagen values. The high $\delta^{15}N$ value (10.1) suggests the goose was feeding in ^{15}N enriched aquatic environments. The ring-necked pheasant's carbon isotope values ($\delta^{15}N$= 8.2°/$_{oo}$ and $\delta^{13}C$= -11.1°/$_{oo}$) show a dominant C_4 diet. These values are typical of a corn-based diet.

All prehistoric animals, except one bison, had very negative $\delta^{13}C$ values, indicating primarily C_3 food sources (Table 2). The four bison values are especially interesting because the positive -11.0°/$_{oo}$ is near the value we might expect of a grazer on a C_4-dominated prairie. However, very negative values of the remaining three are impossible for a prairie in this region. Reexamination by a physical anthropologist confirms these toe bones as bison. These bones, however, were found together and this specific bone type was involved with trade. We suspect, therefore, that these specimens represent trade items from the east where those signals would be possible.

The high $\delta^{15}N$ values suggest that for raccoon, dog, and golden eagle a significant part of the diet was animal meat (Ambrose, 1987). The golden eagle, a carnivore, has a negative $\delta^{13}C$ value -19.0°/$_{oo}$, probably due to a high amount of upper trophic level fish in its diet, which would also account for its fairly high $\delta^{15}N$. Porcupines have an average $\delta^{15}N$ value of 6.2°/$_{oo}$, indicating they are herbivorous or slightly omnivorous. Ducks have a very negative $\delta^{13}C$ value suggesting an almost total C_3 diet, and the intermediate $\delta^{15}N$ value (approximately 7.5°/$_{oo}$) indicates that phytoplankton or macrophytes were important (Schoeninger and DeNiro, 1984). Turkeys also have $\delta^{13}C$ values that indicate an almost total C_3 diet. The wolf had a positive $\delta^{13}C$ value of -12.5°/$_{oo}$ and a $\delta^{15}N$ of 8.9°/$_{oo}$. This may reflect a diet of prey that consumed mainly C_4 grasses.

Analyses of modern and prehistoric collagen for deer and raccoon yield interesting comparisons (Figure 1), even though our sample size is severely limited. We suggest, however, that the correlation of the trends with time indicate increase of the availability of C_4 plants with time. Modern deer collagen values differ between the sexes. The average collagen value for prehistoric deer is similar to that of the modern buck deer, indicating similar diets and habits. Modern doe,

Table 2. Isotopic values (°/$_{oo}$) for all prehistoric wildlife samples.

Scientific Name	Common Name	$\delta^{13}C$ Collagen	$\delta^{13}C$ Apatite	$\delta^{15}N$	C:N
Odocoileus sp.	Deer	-20.1	-10.6	6.2	3.2
Odocoileus sp.	Deer	-20.2	-9.7	5.9	3.2
Odocoileus sp.	Deer	-20.9	-16.1	6.1	3.2
Odocoileus sp.	Deer	-21.3	-14.4	4.9	3.2
Odocoileus sp.	Deer	-22.7	-13.0	3.4	3.2
Odocoileus sp.	Deer	-20.4	-11.9	5.2	3.3
Odocoileus sp.	Deer	-16.6	*	6.1	3.3
Odocoileus sp.	Deer	-20.8	*	5.0	3.1
Odocoileus sp.	Deer	-20.8	-9.4	6.5	3.3
Odocoileus sp.	Deer	-20.7	-12.7	6.3	3.3
Odocoileus sp.	Deer	-21.1	-11.4	6.0	3.2
Bison bison	Bison	-20.2	-9.6	6.7	3.2
Bison bison	Bison	-19.2	-10.0	5.8	3.1
Bison bison	Bison	-18.7	-9.8	7.9	3.2
Bison bison	Bison	-11.0	*	5.9	3.2
Procyon lotor	Raccoon	-18.8	*	8.8	3.1
Procyon lotor	Raccoon	-21.8	*	7.5	3.3
Procyon lotor	Raccoon	-21.3	-10.5	8.4	3.2
Procyon lotor	Raccoon	-19.4	*	9.7	3.3
Procyon lotor	Raccoon	-19.7	-14.2	8.8	3.2
Procyon lotor	Raccoon	-20.5	*	8.6	3.2
Erithizon dorsatum	Porcupine	-21.6	-15.0	8.3	3.2
Erithizon dorsatum	Porcupine	-20.4	-15.1	5.1	3.2
Erithizon dorsatum	Porcupine	-20.3	-10.6	5.3	3.2
Canis lupis	Wolf	-12.5	-7.8	8.9	3.2
Canis familiaris	Dog	-18.5	*	9.4	3.1
Canis familiaris	Dog	-17.9	*	9.0	3.1
Canis familiaris	Dog	-18.5	*	10.0	3.1
Canis familiaris	Dog	-18.3	*	8.8	3.2
Canis familiaris	Dog	-18.0	*	9.0	3.1
Canis familiaris	Dog	-18.5	*	9.5	3.2
Canis familiaris	Dog	-17.9	*	9.2	3.2
Canis familiaris	Dog	-18.5	*	9.5	3.1
Lepisosteus osseus	Fish	-19.4	*	10.8	3.1
Lepisosteus plastomas	Fish	-18.2	*	10.1	3.1
Catostomidae sp.	Sucker Fish	-23.0	*	8.8	3.2
Catostomidae sp.	Sucker Fish	-22.2	*	8.4	3.2
Meleagrididae sp.	Turkey	-19.7	-14.4	7.2	2.9
Meleagrididae sp.	Turkey	-21.5	*	3.1	3.2
Meleagrididae sp.	Turkey	-21.2	*	3.2	3.2
Meleagrididae sp.	Turkey	-20.6	-15.2	4.5	3.1
Anas sp.	Duck	-26.1	*	7.3	3.2
Anas sp.	Duck	-20.8	*	7.8	3.2
Aquila sp.	Golden Eagle	-19.0	-14.3	10.4	3.2

on the other hand, exhibit different behavior and have access to abundant C_4 corn resulting in a positive shift of their collagen value. The mean isotopic values for modern and prehistoric raccoon also show a similar shift in diet. The prehistoric raccoon relied heavily on C_3-based vegetation in the absence of widespread corn.

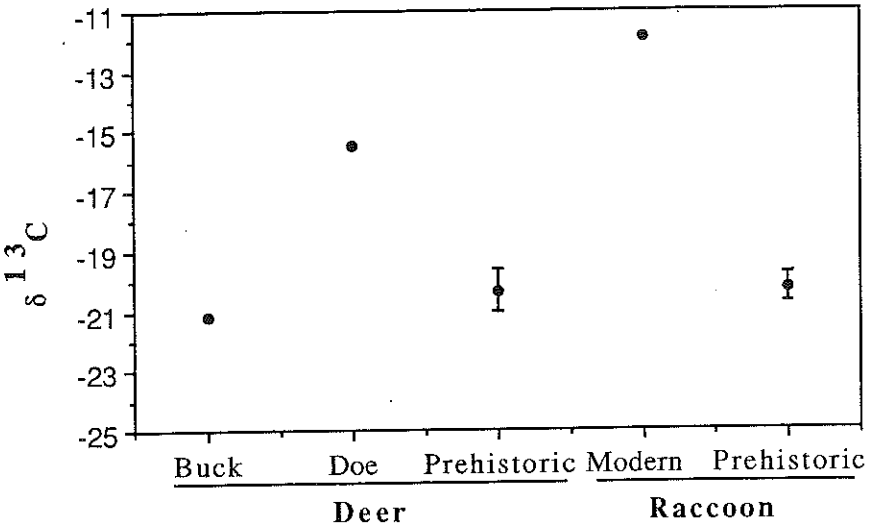

Figure 1. Carbon isotope comparison of modern and prehistoric deer and racoon. Bars indicate standard error.

Analysis of all specimens shows that the prehistoric animals were influenced more by C_3 vegetation than C_4 vegetation (Table 2). The greater dependence of modern animals on C_4 vegetation (Table 1) shows the impact that agriculture has played on the diets of native animals. The use of stable isotopes for modern animals can supplement rumen analysis, fecal analysis, behavioral studies, and habitat analysis to provide a more quantitative understanding of wildlife diet. Stable isotopes in fecal remains have been used to determine seasonal variations in the diets of the buffalo (Tieszen et al., 1983a) as well as herbivore selectivity of plants among various species in Kenya (Tieszen and Imbamba, 1980). Isotopic analysis of fecal samples in a variety of habitats in this region could document seasonal dependencies in various species. Bone collagen or muscle samples, on the other hand, could be used to integrate longer-term assimilation of plants and could document, quantitatively, the percentage corn consumed by deer or other wildlife.

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